Ligation

1. Check the concentration of DNA fragments and vector which are going to be ligated.
2. Calculate the amount of partA/partB and vector added, based on the fragment length. Note that a ligation using a molar ratio of 1:3-1:5 vector to inserts.
3. Add DNA/buffer and ligase together in the EP tube.
   20.0μL reaction system

   A μL part A
   B μL part B
   VμL vector
   2μL 10x T4 buffer
   1.25μL ddH2O
   1μL T4 ligase

   --------**20.0μL** Total
4. Mix the reaction by pipetting up and down Gently and microfuge briefly.
5. Incubate at 16°C 20min (high concentration T4 DNA Ligase can be used in a 10 minute ligation)
6. Chill on ice and transform10-20 μl of the reaction into 50 μl competent cells.